学位論文

論文題目

Intratumoral injection of IFN-β induces chemokine production in melanoma and augments the therapeutic efficacy of anti-PD-L1 mAb

（インターフェロンβの腫瘍内局注は悪性黒色腫のケモカイン産生を誘導し、抗PD-L1抗体の治療効果を増強する）

上原治朗

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Intratumoral injection of IFN-β induces chemokine production in melanoma and augments the therapeutic efficacy of anti-PD-L1 mAb

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ABSTRACT

Despite recent advances in treatment for melanoma patients through using immune checkpoint inhibitors, these monotherapies have limitations and additional treatments have been explored. Type I IFNs have been used to treat melanoma and possess immunomodulatory effects including enhancement of T-cell infiltration. T-cell plays a critical role in immune checkpoint therapies via restoration of effector functions and tumor infiltration by T-cells predicts longer survival in a variety of cancer types. Moreover, tumor-infiltrating T-cells are associated with the expression of chemokines such as CCL5 and CXCR3 ligands in tumor tissues. We therefore investigated whether intratumoral injection of IFN-β induces the expression of CCL5 and CXCR3 ligands in melanoma cells and has additional antitumor effects when combined with anti-PD-L1 mAb treatment. IFN-β treatment enhanced $CD8^+$ T-cell infiltration into tumors and CCL5 and CXCR3 ligand expression. *In vivo* studies using a mouse model showed that monotherapy with IFN-β, but not with anti-PD-L1 mAb, inhibited tumor growth in comparison to control. However, the therapeutic efficacy of IFN-β was significantly enhanced by the addition of anti-PD-L1 mAb. This antitumor response of combination therapy was abrogated by anti-CD8 mAb and IFN-β augmented the neoantigen-specific T-cell response of anti-PD-L1 mAb. Our findings suggest that IFN-β induces the expression of CCL5 and CXCR3 ligands in melanoma, which could play a role in T-cell recruitment, and enhances the efficacy of anti-PD-L1 mAb treatment in a CD8-dependent manner.

1. Introduction

Melanoma is the most aggressive form of skin cancer and its incidence is increasing. However, the majority of melanomas are diagnosed with localized disease and the 5-year relative survival is 98%. In contrast, 4% of cases are diagnosed at a distant stage with a 5-year survival rate of 17% and account for the vast majority of skin cancer-related deaths [1]. The treatment options for patients with metastatic melanoma have been very limited and have provided little survival benefit for decades despite advances in cancer therapy [2,3]. In 2011, anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) was approved by the US Food and Drug Administration for the treatment of metastatic melanoma based on a phase III study that showed increased overall survival [4]. Since then, a new era of cancer immunotherapy has begun. CTLA-4 is a key negative regulator of T-cells and inhibition of CTLA-4 signaling enhances T-cell activation, which leads to enhanced antitumor immune responses [4,5]. Similarly, programmed death receptor 1 (PD)-1/PD-ligand 1 (PD-L1) blockade functions as an immune checkpoint inhibitor and improves response rate and survival in metastatic melanoma patients [6,7]. However, the efficacy of these monotherapies is insufficient; less than half of the patients receive a clinical benefit. Hence, additional treatments are required to improve the outcome of patients.

One of the reasons for the failure of cancer immune therapy may be attributed to an insufficient number of effector T-cells in the

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tumor microenvironment. Thus, tumor infiltration with effector T-cells is correlated with better prognosis in several types of cancer including melanoma [8] and detection of tumor-reactive T-cells in PBMCs is not often associated with improved clinical outcomes [9,10]. Type I IFNs, which have been used as an adjuvant therapy for melanoma [11], possess immunomodulatory activities including promotion of the infiltration of lymphocytes into the tumor [12,13]. In addition, CCL5 and CXCR3 ligands: CXCL9-11, play an important role in recruitment of effector T-cells to the tumor site and the expression of these chemokines shows a positive correlation with both increased T-cell infiltration and the survival of patients with cancer [14,15]. In this study, we therefore investigated whether IFN-β induces the expression of CCL5 and CXCR3 ligands in melanoma cells and the efficient antitumor responses when combined with PD-1/PD-L1 blockade.

2. Materials and methods

2.1. Clinical samples

Tumor tissue samples were obtained from patients with metastatic melanoma by surgical resection at the Asahikawa Medical University Hospital. This study was approved by the Asahikawa Medical University Research Ethics Committee and all patients gave written informed consent.

2.2. Cell lines

The human melanoma cell lines 624mel, 697mel, and 888mel were kindly provided by Dr. Kawakami and Dr. Topalian (National Cancer Institute, NIH). SK-MEL-28 human melanoma cell line was purchased from the American Type Culture Collection. B16F10 mouse melanoma cell line was kindly provided by Dr. Kitamura (Hokkaido University).

2.3. Mice

C57BL/6J mice (female, 6-10-week-old) were purchased from Charles River Laboratories Japan, Inc. All mice were maintained and handled according to the protocols approved by the Asahikawa Medical University Institutional Animal Care and Use Committee.

2.4. Ex vivo culture

The cell lines and tumor tissues were cultured in RPMI-1640 medium (Nacalai Tesque) supplemented with 10% fetal bovine serum (Biowest), penicillin (100 U/ml), and streptomycin (100 μg/ml). The resected melanoma tissues obtained from patients were cut into small pieces and stimulated with or without human IFN-β (2000 U/ml, Toray Industries, Inc.). After 24 h of treatment, tissue explants were harvested for determination of mRNA expression. Culture supernatants were collected after 48 h of treatment for measurement of chemokine production. The human melanoma cell lines were cultured with human IFN-β (2000 U/ml, Toray Industries, Inc.) or PBS for 24 h and their mRNA expression and chemokine production were then analyzed. Murine B16F10 cells were stimulated with mouse IFN-β (2000 U/ml, BioLegend) or PBS for 60 h and the expression of cell surface molecules was then analyzed.

2.5. RNA extraction and quantification of mRNA levels

Total RNA was extracted using RNeasy Mini Kit (Qiagen) and was reverse-transcribed to cDNA using PrimeScript 1st strand cDNA Synthesis Kit (Takara Bio). These samples were amplified using *LightCycler 480 Probes Master* (Roche Diagnostics) and TaqMan probes purchased from Applied Biosystems as follows: *CCL5* (Hs00982282_m1), *CXCL9* (Hs00171065_m1), *CXCL10* (Hs01124252_g1), *CXCL11* (Hs04187682_g1), Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*, Hs02758991_g1), *Ccl5* (Mm01302427_m1), *Cxcl9* (Mm00434946_m1), *Cxcl10* (Mm00445235_m1), *Cxcl11* (Mm00444662_m1), and *Gapdh* (Mm99999915_g1). mRNA expression was measured by quantitative real-time PCR using the LightCycler 480 System and Software (Roche Diagnostics). *GAPDH* was used as an internal control and the relative expression of mRNAs was calculated using the ddCt method.

2.6. ELISA

The amounts of chemokines in the supernatant were measured using ELISA MAX™ Deluxe set (CCL5 and CXCL10, BioLegend), ELISA Kit (CXCL9, ThermoFisher Scientific) and LEGEND MAX™ ELISA kit (CXCL11, BioLegend) according to each manufacturer's instructions.

2.7. Immunohistochemistry

Formalin-fixed, paraffin-embedded metastatic melanoma tissue sections were deparaffinized in xylene and hydrated with a graded series of alcohol. Antigen retrieval was performed in a thermal hot pot with TE buffer at 98 °C for 40 min. After blocking endogenous peroxidase activity with 1% H_2O_2 solution in methanol, the sections were blocked with 10% rabbit serum and incubated with mouse anti-human CD8 monoclonal antibody (clone C8/144B, Dako, dilution 1:100) at room temperature for 1 h. Antibody binding was detected with biotinylated rabbit anti-mouse IgG + IgA + IgM antibody (Histofine, Nichirei Bioscience) and peroxidase-conjugated streptavidin (Histofine, Nichirei Bioscience), and was visualized using 3,3'-diaminobenzidine (DAB) substrate (DaKo) followed by counterstaining with hematoxylin. The slides were observed under a light microscope (BX53F, Olympus).

2.8. Flow cytometry

Tumor tissues were resected from B16F10-bearing mice and treated with 2% collagenase type II (Worthington Biochemical Corporation) for 60 min at 37 °C after mincing with a scissors. Isolated tumor-infiltrating lymphocytes (TILs) or IFN-β-stimulated B16F10 cells were stained with fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)-, PE combined with a cyanine dye (PE-Cy7)-, or allophycocyanin (APC)-conjugated mAbs. The following antibodies were purchased from BioLegend: anti-CD3 (145-2C11), anti-CD4 (GK1.5), anti-CD8a (53–6.7), anti-CD45 (30-F11), anti-CD274 (10F.9G2), anti-H-2k^b (AF6-88.5), anti-I-A^b (25-9-17), rat IgG2b,k (RTK4530), and mouse IgG2a,k (MOPC-173). The samples were analyzed using the BD Accuri C6 flow cytometer and software (BD Biosciences).

2.9. Therapeutic studies in a mouse model

C57BL/6J mice were injected intradermally with 5×10^4 B16F10 murine melanoma cells on day 0, and received an intratumoral injection of 5000 U IFN-β (BioLegend) on days 7, 9, and 11 and/or an intraperitoneal injection of 100 μg anti-PD-L1 mAb (clone 10F.9G2, BioLegend) on days 7 and 9. Rat IgG2b isotype control (clone RTK4530, BioLegend) was used for mock treatment. For the CD8$^+$ cell depletion, mice were injected intraperitoneally with 100 μg anti-CD8a mAb (clone 53–6.72, BioXcell) or rat IgG2a isotype control (clone 2A3, BioXcell) on days 6 and 10 after tumor inoculation. Antitumor effect was determined by measuring the tumor size at each time point. Mice were sacrificed when tumors reached

a size of 300 mm^2.

2.10. ELISPOT

Draining lymph node cells (2×10^5) were cocultured with a peptide (3 μg/ml) for 24 h and the number of IFN-γ-producing cells in the culture was measured using ELISpot kit (Mabtech AB) according to the manufacturer's instructions. The following peptides were purchased from Sigma-Aldrich (PEPScreen): Tnpo3 (G504A) VVDRNPQFLDPVLAYLMKGLCEKPLAS, Plod2 (F530V) STANYNTSH LNNDVWQIFENPVDWKEK, and Obsl1 (T1764M) REGVELCPGN-KYEMRRHGTTHSLVIHD. Tnpo3 (G504A) and Plod2 (F530V) are CD4 epitopes, and Obsl1 (T1764M) is a CD8 epitope [16].

2.11. Statistical analyses

GraphPad Prism software (GraphPad Software, Inc.) was used to determine significant differences. Differences between two groups or among multiple groups were assessed by the unpaired *t*-test or the two-way ANOVA test, respectively. The log-rank (Mantel-Cox) test was performed to evaluate significant differences in survival of mice on Kaplan-Meier plots among the groups. Data are presented as mean ± SD or SEM. $P < 0.05$ was considered significant.

3. Results

3.1. IFN-β treatment increases CCL5 and CXCR3 ligands in metastatic melanoma tissues and cell lines

We first examined the effect of IFN-β on chemokine expression in metastatic melanoma tissues obtained from patients using real-time PCR. IFN-β enhanced the mRNA levels of CCL5 and CXCL9-11 in both clinically treated (Fig. 1A) and resected (Fig. 1B) melanoma tissues compared to control treatment. Since these chemokines are known to recruit lymphocytes to the site of inflammation [14,15], we analyzed lymphocyte infiltration into the tumor site using immunohistochemical staining. This staining confirmed that IFN-β treatment in patients with metastatic melanoma increased tumor infiltration by $CD8^+$ cells (Fig. 1C).

We next investigated a possible role of IFN-β in the production of chemokines from melanoma cells. As shown in Fig. 1D, enhanced production of CCL5 and CXCL9-11 was observed in the human melanoma cell lines stimulated with IFN-β compared to controls, although some differences in sensitivity to IFN-β were observed among the cell lines. Furthermore, these melanoma cell lines enhanced mRNA expression of the chemokines CCL5 and CXCL9-11 in response to IFN-β (data not shown). Taken together, these data suggest that IFN-β stimulation induces CCL5 and CXCR3 ligands in melanoma.

3.2. Combination therapy with IFN-β and anti-PD-L1 mAb results in significantly inhibited tumor growth and prolonged survival

To evaluate the therapeutic efficacy of IFN-β against melanoma, B16F10 murine melanoma cells were injected into C57BL/6J mice and established tumors were treated with intratumoral injection of IFN-β. Analysis of the frequency of tumor infiltrating T-cells and mRNA levels in the tumor tissues showed that IFN-β treatment significantly enhanced the accumulation of tumor-infiltrating $CD4^+$ and $CD8^+$ T-cells (Fig. 2A), and the mRNA expression of Ccl5 and Cxcl9-11 compared to controls (Fig. 2B). Furthermore, FACS analysis showed that in addition to increasing MHC class I, IFN-β upregulated the expression of PD-L1 on B16F10 melanoma cells both *in vitro* (Fig. 2C) and *in vivo* (Fig. 2D), whereas there was no change in the levels of MHC class II, suggesting that IFN-β treatment might enhance the therapeutic efficacy of PD-1/PD-L1 blockade. We therefore examined this hypothesis using an *in vivo* mouse model. C57BL/6J mice bearing B16F10 cells received IFN-β intratumorally on days 7, 9, and 11 and/or anti-PD-L1 mAb intraperitoneally on days 7 and 9 after tumor inoculation. The combination of the two agents resulted in significantly inhibited tumor growth and prolonged survival in comparison with each monotherapy and control treatments (Fig. 3A). Monotherapy with IFN-β also enhanced antitumor responses compared to control, but with less efficacy than the combination therapy. On the other hand, treatment with anti-PD-L1 mAb alone failed to suppress tumor growth.

Furthermore, to determine the involvement $CD8^+$ T-cells in the observed antitumor activity of this combination therapy, B16F10-bearing mice received the combination regimen using the same treatment schedule as shown in Fig. 3a combined with an intraperitoneal injection of anti-CD8 or isotype control mAb on days 6 and 10 post-inoculation with B16F10 cells (Fig. 3B). Consistent with the data in Fig. 3A, the combination therapy of IFN-β with anti-PD-L1 mAb promoted antitumor responses in mice given isotype control mAb. However, depletion with anti-CD8 mAb abrogated the effect of the combination therapy, indicating that this therapeutic effect is $CD8^+$ T-cell-dependent.

3.3. IFN-β augmented neoantigen-specific T-cell responses induced by anti-PD-L1 mAb

To gain a better understanding of the functional role of T-cells in the combination therapy, we analyzed the frequency of neoantigen-specific T-cells using ELISPOT assay since T-cell activity towards neoantigens plays an important role in the clinical efficacy of cancer immunotherapies [17]. C57BL/6J mice bearing B16F10 cells were treated with IFN-β and/or anti-PD-L1 mAb on day 7 and tumor-draining lymph node cells were collected on day 10 after tumor inoculation followed by stimulation with peptides derived from B16F10-associated neoantigens [18]. Anti-PD-L1 mAb monotherapy induced Tnpo3 (G504A)-specific T-cell response, however, the combination treatment with IFN-β elicited T-cell responses not only against Tnpo3 (G504A) but also against Plod2 (F530V) and Obsl1 (T1764M) (Fig. 4A and B). There were no significant changes in IFN-γ production in both control and IFN-β monotherapy groups, demonstrating that IFN-β treatment increases the frequency of neoantigen-specific T-cells induced by anti-PD-L1 mAb monotherapy.

4. Discussion

Our data demonstrated that IFN-β induces the expression of CCL5 and CXCR3 ligands in melanoma cells, which could contribute to recruitment of effector T-cells into tumor tissues, and enhances antitumor immunity when combined with anti-PD-L1 mAb treatment via $CD8^+$ T-cell-dependent responses. Since effective cancer immunotherapy is thought to require both infiltration of effector T-cells and abrogation of immunosuppressive effects in the tumor environment, the effect of combined IFN-β and anti-PD-L1 mAb would complement each other and therefore the combined therapy would be a more effective strategy for the treatment of melanoma patients.

Type I IFNs exhibit a variety of biological functions including antiproliferation, antiangiogenesis, proapoptosis, and immunomodulation, and they exert antitumor activity against melanoma [13]. Most clinical studies in melanoma have focused on IFN-α and several studies have shown that IFN-α therapy improved both overall and disease-free survival of patients with advanced melanoma [11]. On the other hands, IFN-β is widely used as an adjuvant for melanoma therapy in Japan and has been shown to prolong

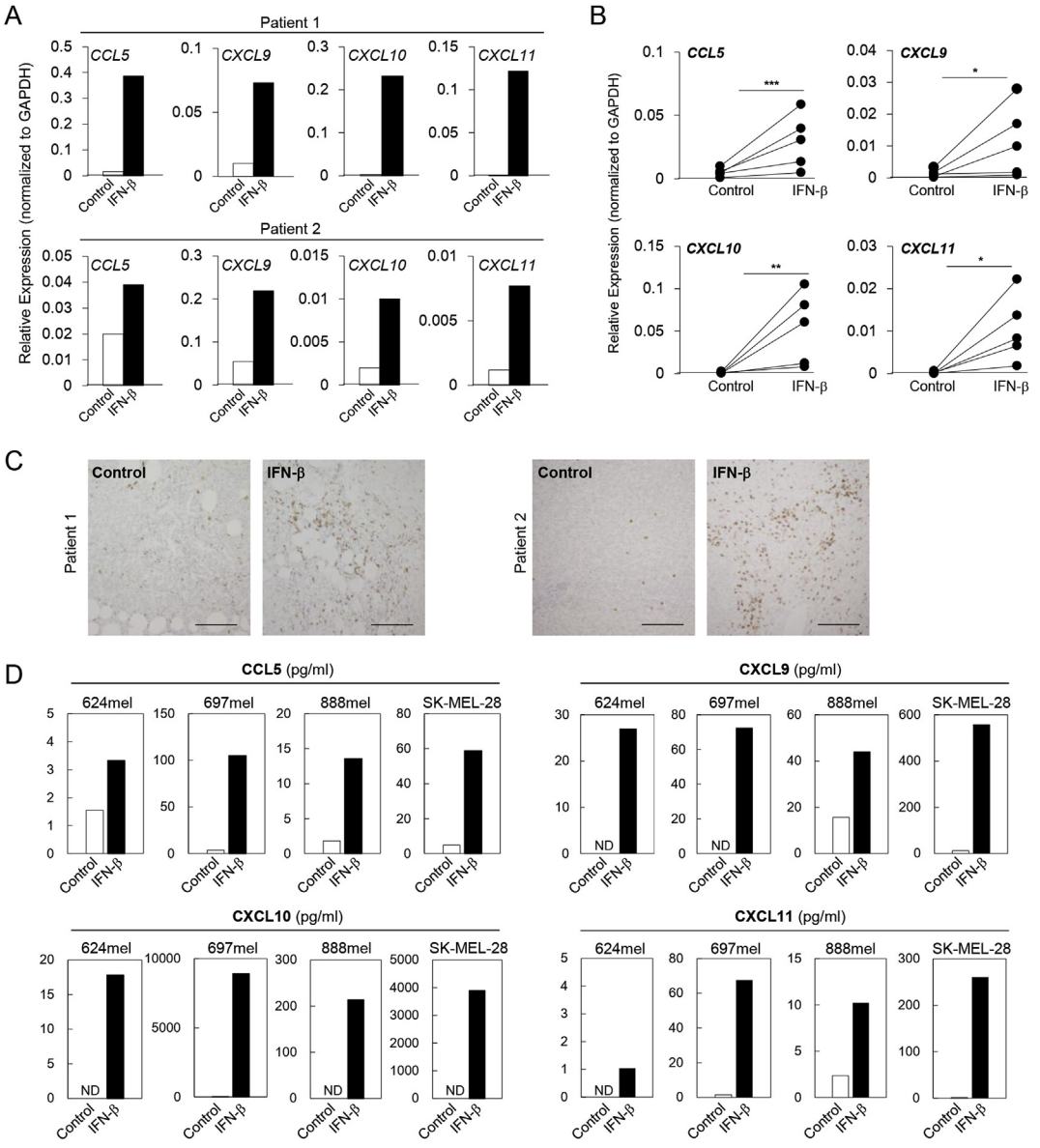

Fig. 1. Elevated expression of CCL5 and CXCR3 ligand chemokines in both melanoma tissues and cell lines in response to IFN-β.
(A) Chemokine expression levels and (C) immunohistochemical staining for CD8 in metastatic melanoma tissues in patient treated with or without IFN-β (n = 2). The scale bars indicate 200 μm. (B) Resected metastatic melanoma tissues from patients (n = 5) were cut into small pieces and incubated with IFN-β or PBS. After 24 h of treatment, chemokine expression levels in cultured tumor explants were analyzed using real-time PCR (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). (D) The human melanoma cell lines were stimulated with IFN-β or PBS for 24 h, and then chemokine production levels in the supernatant were determined using ELISA (ND, not detected). Representative data of two or three independent experiments are shown.

overall and relapse-free survival [19]. Moreover, IFN-β as well as IFN-α increases tumor infiltration by immune cell subsets including $CD4^+$ and $CD8^+$ T-cells [12]. Consistent with these findings, we demonstrated here that IFN-β treatment prolonged survival compared with the control group and increased T-cell recruitment into the tumor tissue of B16F10-bearing mice, which could contribute to the therapeutic efficacy of immune checkpoint inhibitors. We also found that anti-PD-L1 mAb treatment had no effect on the mRNA expression of CCL5, CXCL9, CXCL10, and CXCL11 that was induced by IFN-β monotherapy in B16F10 tumor tissues (data not shown), suggesting that additional treatment with anti-PD-L1 mAb does not influence T-cell infiltration. A previous study demonstrated that peritumorally administered IFN-β enhances the antitumor effect of anti-PD-1 mAb against B16F10 melanoma [20]. Similarly, our data show that combined therapy of IFN-β with anti-PD-L1 mAb inhibits tumor growth in comparison to control and monotherapy groups. Moreover, we have recently reported that intratumoral administration of a ligand for stimulator of IFN genes (STING) results in IFN-β signaling and antitumor effects that are mediated by $CD8^+$ T-cells and macrophages [21]. Hence, STING ligands such as cyclic diguanylate monophosphate or cyclic [G(2′,5′)pA(3′,5′)p] may be used instead of IFN-β.

PD-L1 expression has been observed in many tumors and seems to be correlated with poor prognosis in several cancers including melanoma [22,23]. Conversely, it has been reported that increased expression of PD-L1 appears to be associated with a trend towards better survival in stage III/IV melanoma patients [24]. In addition, higher expression levels of PD-L1 are associated with both elevated CD3 expression and prolonged overall survival in metastatic melanoma [25]. Another study showed that an anti-EGFR-IFNβ fusion protein induces PD-L1 expression on tumor cells and that the combination of this fusion protein with anti-PD-L1 blockade

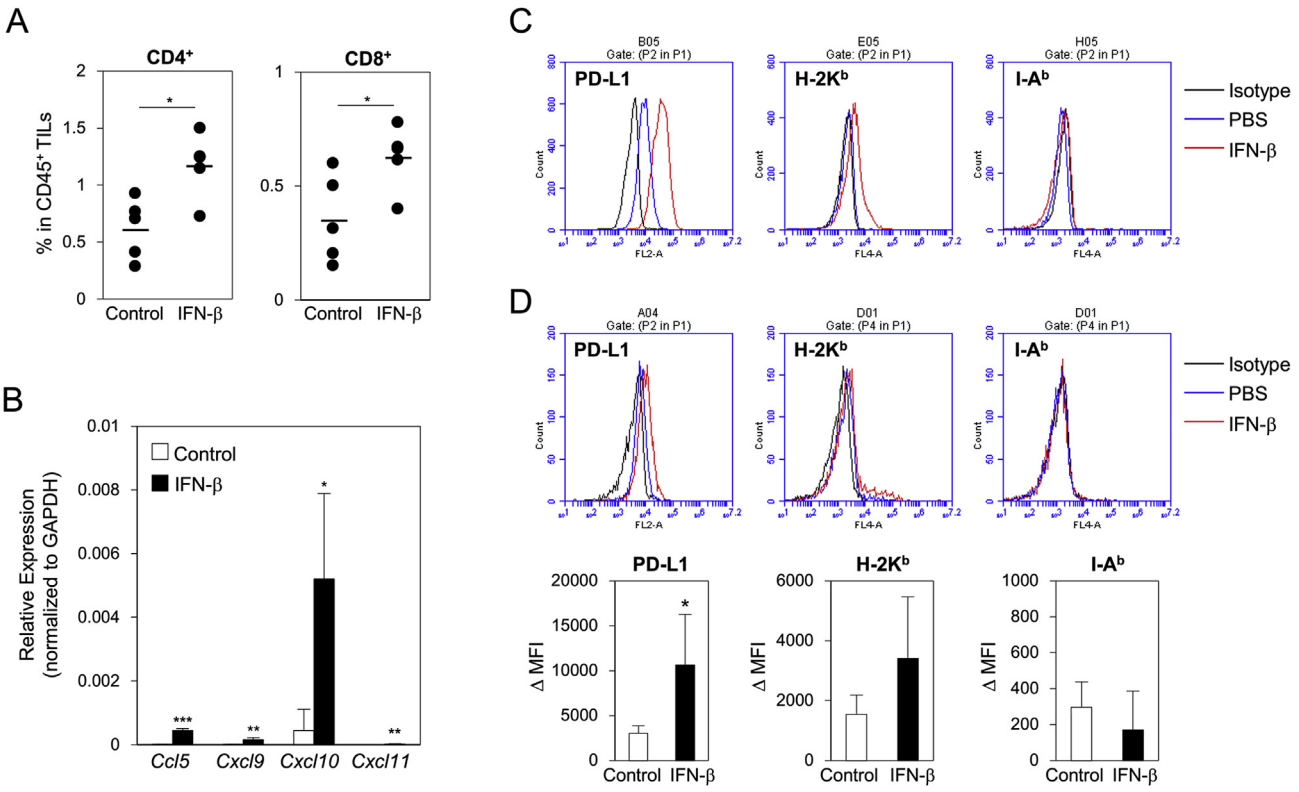

Fig. 2. IFN-β treatment resulted in accumulation of T-cells and expression of chemokines in B16F10 murine melanoma tissues.
C57BL/6J mice were injected with B16F10 melanoma cells on day 0 and intratumorally with IFN-β or PBS on day 7. (A) The frequency of T-cells in TILs on day 8 was analyzed using flow cytometry (n = 5 per group; $^{*}P < 0.05$). (B) After 6 h of treatment, chemokine expression levels in tumor tissues were determined using real-time PCR (n = 4 per group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). (C) B16F10 melanoma cells and (D) B16F10-bearing C57BL/6J mice were treated with IFN-β or PBS *in vitro* and *in vivo* (intratumoral injection), respectively. The expression of cell surface molecules on B16F10 were then measured using flow cytometry at 60 h (C) or 24 h (D) after treatment. ΔMFI means differences of fluorescent intensity between specific antibody and isotype control ($^{*}P < 0.05$). Representative data of three independent experiments are shown (n = 2–3 per group).

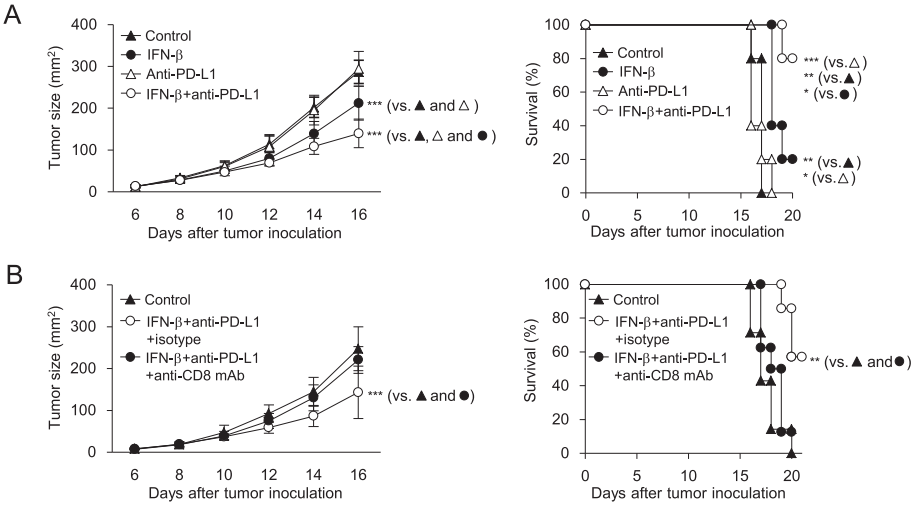

Fig. 3. Combination therapy of IFN-β with anti-PD-L1 mAb led to suppress tumor growth and improve survival in a CD8-dependent manner.
C57BL/6J mice bearing B16F10 melanoma cells were treated with an intratumoral injection of IFN-β on days 7, 9, and 11 and/or an intraperitoneal injection of anti-PD-L1 mAb on days 7 and 9 after tumor inoculation (A). *In vivo* $CD8^{+}$-cell depletion, anti-CD8 mAb was injected intraperitoneally into mice on days 6 and 10 post-inoculation with B16F10 cells (B). Tumor diameter was measured every other day and tumor areas were calculated (n = 7–8 per each group; $^{***}P < 0.001$). Kaplan-Meier plot illustrates the survival (n = 7–8 per each group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

enhances the antitumor effect in a B16-EGFR-SIY model compared to monotherapy [26]. Based on these observations, PD-L1 expression on melanoma cells might provide therapeutic benefit and IFN-β treatment could contribute to this benefit through elevation of both the expression of PD-L1 and MHC class I on B16F10 melanoma cells, although the clinical relevance of PD-L1 expression for efficacy of anti-PD-L1 therapy remains controversial [27,28].

There is a positive correlation between increased T-cell infiltration and the expression levels of CCL5 and CXCR3 ligands not only in melanoma but also in other types of cancer [14,15]. These

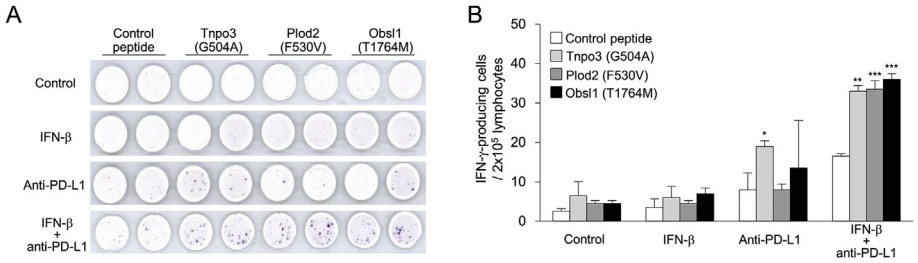

Fig. 4. IFN-β enhanced neoantigen-specific T-cell responses when combined with anti-PD-L1 mAb in the tumor-draining lymph nodes.
C57BL/6J mice bearing B16F10 cells received the combination therapy on day 7 and tumor-draining lymph node cells were collected on day 10 after tumor inoculation. These cells were stimulated with each peptide derived from B16F10-associated neoantigens and IFN-γ production was measured using ELISPOT assay. (A) Representative data of three independent experiments are shown (n = 2 per each group). (B) Bars and error bars indicate the mean and SD, respectively, from three independent experiments (n = 6 per each group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

findings are consistent with our data showing that IFN-β treatment enhances CCL5 and CXCL10 production and tumor infiltration by $CD8^{+}$ cells in human melanoma tissues.

Neoantigens are recognized as non-self because they are derived from nonsynonymous mutations in tumors and are not expressed in normal tissues. Hence, neoantigen-specific T-cells can induce highly specific and effective immune responses without autoimmunity and/or tolerance. In the past several years, many neoantigens have been identified as targets of tumor-specific T cells in both MHC class I and class II epitopes [29], and neoantigen-reactive T cells have been associated with tumor regression and clinical benefit after immune checkpoint inhibitor therapies [17]. A recent study identified immunogenic somatic point mutations in B16F10 melanoma cells [18]. Several of these mutated peptides (neoantigens) elicited IFN-γ production from splenocytes of vaccinated mice and inhibited tumor growth [16,18]. Based on these findings, we evaluated T-cell responses to the neoantigens: Tnpo3 (G504A), Plod2 (F530V), and Obsl1 (T1764M) in IFN-β and/or anti-PD-L1 mAb treated B16F10-bearing mice. Most importantly, we found that combination therapy with IFN-β plus anti-PD-L1 mAb elicited higher frequencies of T-cell responses to neoantigens compared with anti-PD-L1 mAb treatment, while monotherapy with IFN-β and mock treatment failed to induce neoantigen-specific T-cell responses. These results indicate that IFN-β monotherapy may be insufficient to induce effector T-cell function in tumor-draining lymph nodes. IFN-β acts as a double-edged sword because it can enhance not only T-cell migration and tumor apoptosis [13] but also PD-L1 expression in the tumor microenvironment. Hence, increased tumor destruction by IFN-β treatment would enhance tumor-neoantigen load in tumor-draining lymph nodes, and thereby induced neoantigen-specific T-cells when anti-PD-L1 mAb restores the ability to exert effector function. Taken together, the combination therapy of IFN-β and anti-PD-L1 mAb treatment enhances the frequency of neoantigen-specific T-cells with effector functions including both $CD4^{+}$ and $CD8^{+}$ T-cells.

In this study, we demonstrated that IFN-β improves the antitumor activities of PD-1/PD-L1 blockade via two pathways: (1) tumor infiltration by T-cells and (2) induction of neoantigen-specific T-cell responses. Although further investigations are required to determine the mechanisms by which melanoma cells stimulated with IFN-β increase T-cell infiltration into the tumor tissue, IFN-β treatment may be a useful additional therapeutic strategy for melanoma when combined with PD-1/PD-L1 blockade.

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Abbreviations

CCL: C-C motif ligand; **CTLA-4:** cytotoxic T-lymphocyte antigen 4; **CXCL:** C-X-C motif ligand; **CXCR:** C-X-C motif receptor; **IFN:** interferon; **mAb:** monoclonal antibody; **PD-1:** programmed death receptor 1; **PD-L1:** programmed death receptor ligand 1.

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Authors' contributions

JU, TO, AK, and HK contributed to the conception and design. JU contributed to the clinical treatment and acquired the clinical samples. TO and AK performed the experiments and analyzed the data. JU, TO, AK, and HK contributed to the interpretation of data and wrote the manuscript. All authors discussed the data, and read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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